EMBRYONIC DEVELOPMENT OF THE LORDOTIC AND NORMAL GUPPY, LEBISTES RETICULATUS (PETERS) [1]

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Previous studies have shown that the mutation resulting in lordosis of the vertebral column in *Aplocheilus latipes* (Aida, 1930) and in *Lebistes reticulatus* (Kirpichnikov, 1935; Harrison, 1941; Goodrich *et al.*, 1943; Rosenthal and Rosenthal, 1950) behaves as a recessive, autosomal single factor Mendelian character. The lordotic condition becomes progressively more pronounced as the fish ages and may cause swimming activity to be somewhat laborious and erratic. Growth and development of either sex and reproduction in the female do not appear to be affected. However, the fertilizing capacity of sperm from mature mutant males is reduced (Rosenthal, 1951).

Since the spinal curvature is markedly apparent in the new-born young of *Lebistes,* it was of interest to compare the growth and development of the lordotic and normal embryo as an introduction to the study of the biochemistry of the lordotic mutation.

Materials and Methods

Normal fish, obtained from a local tropical fish hatchery,[2] were maintained in the laboratory for variable lengths of time before use. The lordotic fish were raised and maintained under conditions similar to those previously reported (Rosenthal and Rosenthal, 1950; Rosenthal, 1952). As female fish approached parturition, they were isolated to individual jars containing masses of *Ceraptopterus* until the brood was born. The new-born young were removed within 3 to 20 hours, blotted dry on filter paper and the entire brood weighed to the nearest 0.1 mg. The young were then dried to constant weight at 105° C. to determine their water content. The dry residue was next extracted with ten 3-ml. portions of ethyl ether and the fat-free residue was dried an additional 24 hours. The fat content of the material was calculated by difference. A series of embryos [3] was obtained by sacrificing female fish at weekly intervals following the birth of a brood. The fish were killed by dropping them into boiling water since heat coagulation was found to be the best method for removing the ova or embryos from the ovary without breaking them. The ovarian contents were treated in the same manner

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[2] Normal fish and live food such as Tubifex worms were generously supplied by Messrs. William Schaumberg and John Detrich, of Crescent Fish Farms, New Orleans, to whom the author is indebted.

[3] Since the embryo cannot be readily separated from the yolk of the egg, the entire embryo-yolk complex was removed and treated as a unit and this combination of embryo and yolk will be considered interchangeably, for the purposes of this report, as either the ovum, embryo-yolk complex or embryo depending on the data to be discussed.

as new-born young. Embryos or young that were not normally developed were discarded.

For chemical analysis, quantities of dried, fat-free material weighing 15 to 40 mgs. from individual broods of young or groups of ova and embryos were digested by boiling with one ml. concentrated sulfuric acid containing 4 drops of 5% copper sulfate. The digests were completely cleared by the addition of a few drops of 30% hydrogen peroxide. After cooling, the digests were diluted to 10 ml. and suitable aliquots were taken for the determination of calcium by the method of Kramer and Tisdall (1921). Phosphorus was determined on aliquots of the digest by the method of Fiske and Subbarow (1925) and nitrogen by direct nesslerization. Nitrogen values were converted to protein by use of the factor 6.25. Duplicate determinations of calcium agreed within 6% and those for phosphorus and nitrogen within 1%.

Results

A comparison of the data, shown in Table I, on the growth rate, fat and water content of the normal and lordotic *Lebistes* indicates no essential differences with the exception that the lordotic embryo is consistently the smaller. In view of the similarity between the two strains, the data will be discussed together.

Although the variation between groups of embryos is quite large as shown by the standard deviations, it can be seen that the wet weight of maturing ova remains essentially constant for the first seven days but increases rapidly from the time of fertilization until the embryo is born. The increase in weight is due to an in-

Table I

Embryonic growth of Lebistes reticulatus

Age, days	No. of embryos	No. of deter.	Average weight (mgs.)			H_2O % wet wt.	Fat % dry wt.
			Wet	Dry	Fat free		
Normal							
0	185	5	2.96±0.73*	1.46±0.36*	1.14±0.20*	50.6	21.6
7	154	6	2.75±0.40	1.31±0.28	1.01±0.19	53.1	22.5
14	187	6	3.86±0.57	1.33±0.33	1.01±0.31	70.9	24.3
21	178	7	4.95±0.44	1.24±0.25	0.95±0.11	75.0	23.4
Term	279	23	5.64±0.69	1.22±0.20	0.96±0.15	78.4	19.5
Lordotic							
0	47	4	2.06±0.61	1.00±0.28	0.73±0.23	51.6	26.7
7	85	3	2.56±0.12	1.23±0.05	0.94±0.03	52.2	23.5
14	92	8	3.14±0.93	1.29±0.21	1.02±0.19	63.1	21.8
21	50	3	4.06±0.38	1.07±0.18	0.83±0.15	73.8	23.2
Term	108	25	4.53±0.56	0.98±0.20	0.84±0.13	79.2	14.1

* $\sigma = \sqrt{\frac{\Sigma d^2}{N-1}}$.

creasing water content which rises from an initial value of 50% to a final value of 80%. In contrast, the dry and the fat-free dry weight remain essentially constant throughout the development of the embryo. The fat content of the embryo, however, decreases slightly after the young are born. This probably reflects a utilization of stored yolk material during the period immediately following birth, since newly born poeciliid fish have rarely been observed to accept food during the first few hours after birth.

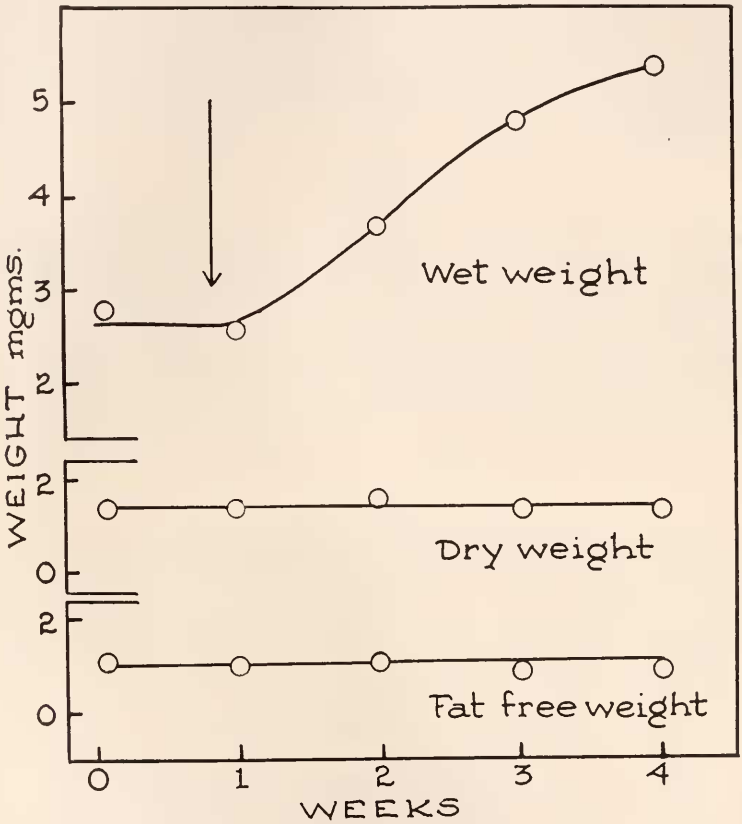

FIGURE 1. Average growth curves for lordotic and normal embryos of *Lebistes* during the brood interval. The arrow indicates the approximate time of fertilization of the ovum. See Table I.

In view of the similarity of the lordotic and normal embryo, the wet, dry and fat-free dry weights have been averaged and plotted against time of development as shown in Figure 1, to demonstrate graphically the growth rate of the *Lebistes* embryo.

calcium and phosphorus content of the dry fat-free tissue during growth and development of the *Lebistes* embryo. It is apparent that the total protein remains

The data presented in Table II show the changes that occur in the nitrogen, essentially constant for both the lordotic and the normal strains although the values

obtained for the lordotic strain are somewhat smaller due to the smaller size of the embryo. The per cent of protein is also the same for both strains and remains essentially constant during gestation. The average total protein of the embryo-yolk complex accounts for 71.1 per cent of the fat-free dry weight (range = 67.1 − 76.1 per cent). The remainder consists of minerals and non-proteinaceous organic matter.

The calcium and phosphorus concentrations of the embryos present a somewhat different picture. During the first three weeks of development, the calcium concentration remains essentially constant but increases rapidly during the last week of gestation. The phosphorus concentration, on the other hand, increases

TABLE II

Embryonic growth of Lebistes reticulatus

Age, days	No. of deter.	Protein		Calcium	Phosphorus	Ca/P
		Total mgs.	% Fat-free dry weight			
			Normal			
0	4	0.77	67.7±3.7*	0.62±0.21*	1.18±0.07*	0.43
7	5	0.71	70.9±5.0	0.81±0.18	1.50±0.08	0.54
14	6	0.70	69.7±3.2	0.76±0.06	1.52±0.07	0.50
21	6	0.70	73.3±2.1	1.08±0.29	1.60±0.60	0.67
Term	12	0.70	73.3±3.9	2.24±0.40	1.90±0.12	1.17
			Lordotic			
0	2	0.50	68.6±4.3	0.95±0.05	1.35±0.05	0.70
7	3	0.65	68.8±0.8	0.73±0.04	1.46±0.05	0.50
14	4	0.74	72.8±1.8	0.97±0.12	1.49±0.08	0.65
21	3	0.63	75.8±4.3	0.93±0.12	1.66±0.09	0.58
Term	8	0.64	76.1±7.9	2.58±0.51	1.92±0.24	1.37

$$^{*}\sigma = \sqrt{\frac{\Sigma d^2}{N-1}}.$$

slightly throughout the brood interval. It can be seen that the Ca/P increases only slightly during the early phases of gestation but increases rapidly during the last week preceding birth. The increasing calcium content is presumably associated with the incorporation of mineral elements in the osseous tissues of the body.

Since no essential differences between the lordotic and normal strains are evident, the data in Table II have been averaged for graphic presentation (Fig. 2).

DISCUSSION

The interval between the birth of successive broods of young for most poeciliid fish in general and *Lebistes* in particular approximates a period of 28 to 30 days. A brood interval may be divided into two separate phases. The first phase consists of 5 to 7 days in which the ova mature to a fertilizable stage (Hopper, 1943;

Rosenthal, 1952; Turner, 1937). The remaining 23 to 25 days, the second phase, may be considered as the period of gestation. The cyclical brood production makes it possible to obtain a graded series of embryos in similar stages of development.

It is apparent from these data that no essential differences exist in the rate of growth of the lordotic and normal *Lebistes* embryo as determined by the methods used. However, Harrison (1941) analyzed the total body calcium of adult

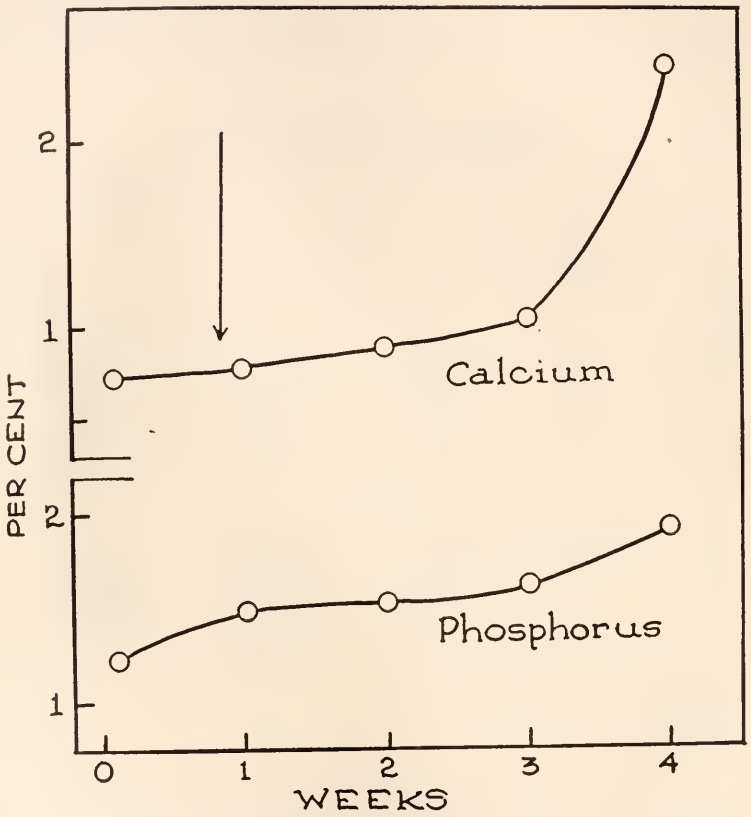

FIGURE 2. Average incorporation of calcium and phosphorus for lordotic and normal *Lebistes* during the brood interval. The arrow indicates the approximate time of fertilization of the ovum. See Table II.

mutant and normal *Lebistes* and found that the mutant strain had a higher calcium content. Additional data (in manuscript) obtained in this laboratory indicate that the vertebral column of the lordotic strain contains about 20% more calcium than the normal.

Scrimshaw (1944; 1945) has studied extensively the embryonic growth pattern of various members of the family Poeciliidae and has concluded, on the basis of the constant dry weight of the embryo yolk complex, that an exchange of nutritive material must occur between the embryo and the tissues of the parent to replace

materials lost through metabolic and excretory processes. The increasing calcium and phosphorus concentrations of the growing embryo, as shown in the present report, indicate that certain materials are obtained from the parent. It is conceivable that organic compounds can also diffuse across the ovarian membranes and, likewise, metabolic products formed during embryogenesis may diffuse through these membranes to be excreted by the parent.

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Summary

1. No differences could be established in the embryonic development of the lordotic and normal *Lebistes*.

2. The water content of developing embryos increases during gestation, but the dry and fat-free dry weights remain constant as does the protein concentration.

3. The calcium concentration, associated with calcification of osseous tissue, increases markedly during the last week of gestation while the phosphorus concentration increases only slightly during the entire brood interval.

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